



12-22-06

AF ZTW

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 748266222 US, in an envelope addressed to: MS Appeal Brief-Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: December 21, 2006

Signature:

Loretta Kavanagh
Loretta Kavanagh

Docket No.: 600-1-081CON
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Ralph Steinman *et al.*

Application No.: 09/586,704

Art Unit: 1644

Filed: June 5, 2000

Examiner: Ronald B. Schwadron

For: IDENTIFICATION OF DEC, A RECEPTOR
WITH C-TYPE LECTIN DOMAINS, NUCLEIC
ACID ENCODING DEC, AND USES
THEREOF

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

As indicated in the Notice of Appeal filed on June 28, 2006, Appellants hereby appeal the final decision of the Examiner in the above-identified application rejecting the subject matter of the pending claims. For the reasons set forth in this brief, Appellants respectfully request the Board of Patent Appeals and Interferences to reverse the Examiner's final rejection of the claimed subject matter. As Applicants are filing this Appeal Brief within the one month time period for response as noted in the Notice of Panel Decision Pre-Appeal Request, no fees are believed to be due. However, if this is in error, authorization is hereby given to charge any fees to deposit account number 11-1153.

BEST AVAILABLE COPY

I. REAL PARTY IN INTEREST

The real party in interest in the above-identified application is Rockefeller University, the assignee of the application.

II. RELATED APPEALS AND INTERFERENCES

A Notice of Appeal and related Pre-Appeal Brief Request for Review were filed on June 28, 2006 in a Continuation-in-Part application, U.S.S.N.: 09/925,284, having a filing date of August 9, 2001. No related interferences are known to Appellants, which will directly affect, or be directly affected by, or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 22-23, 26-30, and 35-45 are pending in this application. Claims 22-23 and 29-30 have been withdrawn from consideration.

Claims 26-28 and 35-45 are on appeal and are set forth in the Claims Appendix (Appendix A).

IV. STATUS OF THE AMENDMENTS

A Notice of Appeal was filed June 28, 2006. All prior amendments have been entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Claims 26-28

The claims on appeal are drawn to a vaccine for inducing an immune response comprising an antigen conjugated to an anti-human DEC-205 antibody or an anti-murine DEC-205 antibody reactive with a human DEC-205 protein comprising the human DEC-205 protein as set forth in SEQ ID NO: 1. The amino acid sequence of SEQ ID NO: 1 corresponds to a partial (C-terminal) sequence of human DEC-205 (see, for example, page 3, lines 15-19; page 5, lines 13-23; page 7, lines 1-12; page 10, line 20 through page 11, line 14; page 55, line 30 through page 56, line 23; page 62, line 26 to page 63, line 15 and page 67, lines 15-21 of the specification as originally filed).

Claims 35-39

The claims on appeal are further drawn to a vaccine for inducing an immune response comprising an antigen conjugated to an anti-human DEC-205 antibody (claim 35) or an anti-mouse DEC-205 antibody (claim 36), wherein the antibody is reactive with the amino acid sequence as set forth in SEQ ID NO: 1. As noted above, the amino acid sequence of SEQ ID NO: 1 corresponds to a partial (C-terminal) sequence of human DEC-205 (see, for example, page 3, lines 15-19; page 5, lines 13-23; page 7, lines 1-12; page 10, line 20 through page 11, line 14; page 55, line 30 through page 56, line 23; page 62, line 26 to page 63, line 15 and page 67, lines 15-21 of the specification as originally filed).

Claims 40-45

The claims on appeal are also drawn to a vaccine for inducing an immune response comprising an antigen conjugated to an antibody which binds mouse DEC-205 having the amino acid sequence of SEQ ID NO: 3, wherein the antibody cross-reacts with human DEC-205. The amino acid sequence of SEQ ID NO: 3 corresponds to the full-length sequence of mouse DEC-205 (see, for example, page 5, lines 13-23; page 7, lines 1-12; page 10, line 20 through page 11, line 14; page 42, lines 24-31; page 55, line 30 through page 56, line 23; page 62, line 26 to page 63, line 15 and page 67, lines 15-21 of the specification as originally filed).

Please also see the Substitute Sequence Listing submitted in the present application on December 22, 2005, which identifies the full length mouse DEC-205 sequence as SEQ ID NO: 3.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellants present the following issue for review:

1. Whether claims 26-28 and 35-45 are properly rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.
2. Whether claims 26-28 and 35-39 are properly rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

VII. ARGUMENTS

A. Summary of Examiner's Rejection of Claims 26-28 and 35-45 Under 35 U.S.C. § 112, First Paragraph, as Failing to Comply with the Written Description Requirement

The Examiner has rejected claims 26-28 and 35-45 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. In particular, the Examiner asserts that the specification does not provide adequate written description for the claimed invention because, while the specification discloses the full length sequence of murine DEC-205 protein, it only discloses a partial sequence for human DEC-205. The Examiner asserts that, because human DEC-205 is approximately 1800 amino acids in length, the recitation in the claim of a 30 or 25 amino acid sequence derived from human DEC-205 does not provide adequate written description of a molecule that is almost 1800 amino acids in length. The Examiner further asserts that the claims encompass antibodies that bind any immunogenic epitope on the approximately 1775 undisclosed amino acids of DEC-205, and that the term human DEC-205 presumably encompasses full length human DEC-205, as well as undescribed mutants and alleles of human DEC-205.

B. Appellants' Response

1. Each Independent Claim Requires Separate Consideration

Appellants respectfully disagree with the Examiner's rejection. As a preliminary matter, the scope of claims 26-28 and 35-45 varies and, as such, the assertions made by the Examiner are not equally applicable to all of these claims.

Specifically, contrary to the Examiner's opinion that the claimed antibody conjugates do not bind to any specific epitope of human DEC-205, claims 35-39 are drawn to antibody conjugates which do, indeed, bind to a particular epitope of human DEC-205, namely the C-terminal sequence (SEQ ID NO: 1).

Similarly, that the present specification teaches a partial human DEC-205 sequence is also irrelevant with respect to claims 40-45, since these claims are drawn to a vaccine comprising an antigen conjugated to an antibody that binds to *full length murine DEC-205 protein* (SEQ ID NO: 3). Thus, the Examiner's statement that the antibody conjugates of claims 40-45 bind to "undisclosed amino acids of DEC 205" is incorrect. Indeed, the full length sequence of murine DEC 205 is explicitly provided in

the present application as SEQ ID NO: 3. Moreover, while the antibody conjugates of claims 40-45 also cross-react with human DEC-205, the epitopes of human DEC-205 that the conjugates bind to are thus, by definition, shared with (*i.e.*, cross-reactive with) murine DEC-205. As such, the sequence of these epitopes is provided as part of the full length murine DEC-205 sequence recited in the claims (SEQ ID NO: 3).

For at least the reasons above, the reasons provided by the Examiner for rejecting claims 35-45 as lacking written description under 35 U.S.C. §112, first paragraph, do not apply or support the rejection.

Finally, with respect to claims 26-28, drawn to a vaccine comprising an antigen conjugated to an antibody that binds to human DEC-205 protein comprising the partial amino acid sequence of SEQ ID NO: 1, Appellants respectfully submit that while Appellants' specification does not recite the full length human DEC-205 sequence, or the sequence of each and every variant of human DEC-205, this does not *de facto* mean that the pending claims fail to comply with the written description requirement. Importantly, it is well-established that the written description standard is not a bright line test, but instead takes into consideration a number of different factors. As discussed in detail below, Appellants' disclosure of the partial human DEC-205 sequence and the full length murine DEC-205 sequence, in combination with knowledge available in the art, were sufficient to demonstrate to one of ordinary skill that they had full possession of the complete human DEC-205 protein, and antibody conjugates against the protein, at the time the present application was filed.

2. The Descriptive Text Needed to Satisfy the Written Description Standard Must be Considered in Relation to the Scientific Knowledge in Existence at the time of the Invention, the Skill in the Art, and Correlation of a Disclosed Function to a Known Structure

The mere fact that Appellants' specification does not recite the full length human DEC-205 sequence does not alone mean that any of the claims on appeal fail to comply with the written description requirement.

Moreover, Appellants respectfully disagree with the Examiner's assertion that the decision in *Capon v. Eshhar* (418 F.3d 1349, 1357 (Fed. Cir. 2005)) "is not relevant to the claims under consideration." While the claims on appeal may differ from the claims on appeal in *Capon v. Eshhar*, the Court took considerable effort to lay out the underlying framework for determining written description in other cases moving

forward, and to clarify that written description, like enablement, must be determined on a case by case basis. Specifically, the standard for meeting the written description requirement and showing possession of the claimed invention, as articulated by *Capon v. Eshhar*, differs for every patent specification depending upon a number of factors, including the scientific knowledge in existence at the time of the invention, the skill in the art, the predictability of the claimed subject matter, and correlation of a described function to a known structure. Again, Appellants do not argue that the claims at issue in *Capon v. Eshhar* were the same as in the present case, rather that the written description standard articulated by the Court, when applied in the present case, is fully satisfied.

Specifically, as discussed further below, the maturity of the science and skill in the art at the filing date of the present invention were such that one of ordinary skill could predictably obtain full-length proteins, such as DEC-205, based on partial sequences, as well as predictably obtain antibodies against the full-length protein (or any region or variants of the protein). As such, Appellants teachings in the specification, combined with the knowledge available in the art, demonstrate that Appellants were in full possession of the presently claimed invention at the time of filing.

3. Isolation and Cloning of Proteins, and Generation of Antibodies Were Highly Mature Technologies at the Time of the Present Invention

Indeed, at the filing date of the present application (*i.e.*, in 1995), technologies for isolating, characterizing and cloning proteins were highly developed, as were technologies for generating antibodies against such proteins. For example, several well known techniques were available for cloning proteins, including human DEC-205, based on a given partial amino acid sequence of the protein (see, for example, page 20, line 30 through page 21, lines 1-19; as well as page 25, lines 25-31 through page 31, lines 1-16 of the present application). Additionally, techniques for expressing cloned proteins (see, for example, page 31, lines 18-31 through page 35, lines 1-30 of the present application) and for generating antibodies against the proteins were equally well known (see, for example, page 42, lines 23-31 through page 45, lines 1-19, and particularly page 42, lines 28-31 in the present application). Once armed with a partial amino acid (*i.e.*, a peptide derived from a given protein), it was also well within the

skill of the art to use these techniques to generate antibodies against such peptides and to isolate the full-length protein from its natural source.

Appellants specifically illustrated this in relation to mouse DEC-205. In particular, Appellants successfully isolated and characterize full-length mouse DEC-205 from whole murine thymus using mAb NLDC-145, an anti-mouse DEC-205 antibody (see page 63 of the present application). Additionally, Appellants successfully raised antibodies against N-terminal peptides from mouse DEC-205 protein (see, for example, page 62, lines 26-32 and page 63, lines 1-15 of the present application). This provides *clear evidence* that the partial human DEC-205 sequence described in the present disclosure put Appellants in possession of the complete DEC-205 protein and antibodies against the protein.

Additionally, in the present application, Appellants teach a partial (C-terminal) sequence (SEQ ID NO: 1) of human DEC-205 protein. Appellants further teach the highly homologous full-length sequence of mouse DEC-205 protein (SEQ ID NO: 3), along with an in-depth characterization of this protein (including its ability to deliver antigen to an active antigen processing compartment of dendritic cells). Appellants also describe well-known techniques for cloning proteins (including human DEC-205) based on a given partial amino acid sequence of the protein, expressing cloned proteins and generating antibodies against the proteins. Based on these teachings, it was well within the skill of the art to have generated anti-DEC-205 antibodies. It was also well within the skill in the art to have generated full-length human DEC-205 protein, as well as variants of the human DEC-205 protein.

In fact, as evidenced by the Declaration by Dr. Michel Nussenzweig (Appendix B) and related publications submitted with Appellants' Amendment and Response dated January 3, 2005, the cloning techniques and techniques for generating antibodies described in the specification were ultimately successfully used to clone and isolate human DEC-205 and to produce antibodies against full-length human DEC-205. This provides clear evidence that Appellants were in fact indeed in possession of the claimed invention based on the descriptive text provided within the four corners of Appellants' originally filed disclosure.

4. The Structure and Function of Human DEC-205 Correlates to the Structure and Function of Mouse DEC-205 Protein

Finally, the Written Description requirement may be satisfied if the disclosed function of the claimed invention sufficiently correlates to a particular, known structure. In the present case, the structure and function of human DEC-205 clearly correlates to that of mouse DEC-205, the characteristics of which (including full-length sequence) are described in detail in the present disclosure. Accordingly, the fact that Appellants provide an in-depth characterization of mouse DEC-205, including its full-length sequence, which correlates to human DEC-205, provides further basis for fully meeting the Written Description requirement.

In sum, the teachings set forth in Appellants' specification, in combination with the high level of skill and knowledge in the art at the time of the invention, and the proven predictability of the technologies involved in the invention, clearly satisfies the standard for Written Description according to the guidelines articulated by the CAFC in *Capon v. Eshhar* (CAFC 2005), and demonstrates possession of the claimed invention.

C. Summary of Examiner's Rejection of Claims 26-28 and 35-39 Under 35 U.S.C. § 112, First Paragraph, as Failing to Comply with the Written Description Requirement

The Examiner has rejected claims 26-28 and 35-39 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. In particular, the Examiner alleges that there is no support in the specification for a human DEC-205 protein comprising an amino acid sequence as set forth in SEQ ID NO: 1. The Examiner further asserts that, although the specification teaches that SEQ ID NO: 1 is a peptide derived from DEC-205, there is no support for a DEC-205 protein comprising the peptide wherein the molecule could have any amino acids in association with the aforementioned sequences recited in the claim.

D. Appellants' Response

As an initial point, it is unclear to Appellants, based on the Examiner's comments, what the distinction is between the former 35 U.S.C. § 112, first paragraph, rejection of claims 26-28 and 35-45, and the present § 112, first paragraph, rejection of claims 26-28 and 35-39. Indeed, both rejections appear to be based on the same

premise, *i.e.*, that the claims lack written description because the specification teaches a partial human DEC 205 sequence. Appellants note, however, that the former rejection has been applied to claims 26-28 and 35-45, whereas the present rejection has been applied only to claims 26-28 and 35-39.

Accordingly, with respect to claims 35-39, Appellants again respectfully note that these claims are drawn to a vaccine that employs antibody conjugates defined as binding to a *particular* epitope on human DEC 205, the sequence of which is explicitly taught in the application (SEQ ID NO: 1, not SEQ ID NO: 7, as noted by the Examiner). Therefore, the Examiner's assertion that the specification fails to provide support for a human DEC 205 protein comprising the partial sequence of SEQ ID NO: 1 does not provide a basis for rejecting claims 35-39 for lack of written description.

Moreover, for the many reasons discussed above in Section B, Appellants respectfully submit that the specification does indeed provide full support for a human DEC 205 protein comprising SEQ ID NO: 1, as recited in claims 26-28. Again, the mere fact that the disclosure teaches partial sequences for human DEC 205 does not alone mean that the claims covering antibody conjugates which bind to human DEC 205 comprising such sequences lack written description. Whether claims 26-28 comply with § 112, first paragraph, depends on a variety of factors, as discussed above in relation to the previous rejection (Section B). When applied in the present case, given the teachings in Appellants' specification, in combination with the skill and knowledge available in the art at the time the present application was filed, clearly demonstrate that Appellants possessed the complete human DEC-205 protein recited in claims 26-28.

As previously discussed in detail, Appellants teach the partial C-terminal sequence of human DEC-205 (SEQ ID NO: 1). Based on this partial amino acid sequence, it was well within the skill of the art to have used known techniques to generate antibodies against this peptide, and to have predictably isolated the full-length protein or variants from its natural source. In fact, the maturity of the science and skill in the art at the time of the present invention were such that those of ordinary skill in the art were routinely obtaining full-length proteins based on partial sequences, as well as predictably obtaining antibodies against such full-length proteins. This is specifically attested to in the Declaration submitted by Declaration by Dr. Michel Nussenzweig (Appendix B). Further, the fact that Appellants provide an in-depth characterization of mouse DEC-205, including its full-length sequence, which

correlates to human DEC-205, provides further basis for fully meeting the Written Description requirement.


In sum, for at least the foregoing reasons, claims 26-28 and 35-39 fully comply with 35 U.S.C. § 112, first paragraph.

VIII. CONCLUSION

Appellants submit that claims 26-28 and 35-45 comply with the written description requirement under 35 U.S.C. § 112, first paragraph, and respectfully request that the Board reverse the rejection of claims 26-28 and 35-45 for the reasons set forth above.

Dated: 12/21/06

Respectfully submitted,



Veronica Mallon, Ph.D.
Agent for Appellants
Registration No. 52,491
KLAUBER & JACKSON
411 Hackensack Avenue
Hackensack, NJ 07601
(201) 487-5800

CLAIMS APPENDIX A

26. (Previously presented) A vaccine for inducing an immune response comprising an antigen conjugated to an anti-human Dendritic and Epithelial Cell 205 (DEC-205) antibody or an anti-murine DEC-205 antibody reactive with a human DEC-205 protein, said human DEC-205 protein comprising an amino acid sequence as set forth in SEQ ID 1.

27. (Previously presented) The vaccine of claim 26, wherein the antigen is selected from the group consisting of a virus, a bacterium, a parasite, and a tumor.

28. (Original) The vaccine of claim 26, wherein the immune stimulator is selected from the group consisting of a cytokine, a lymphokine, and an adjuvant.

35. (Previously Presented) A vaccine for inducing an immune response comprising an antigen conjugated to an anti-human Dendritic and Epithelial Cell 205 (DEC-205) antibody, wherein the antibody is reactive with the amino acid sequence as set forth in SEQ ID NO: 1.

36. (Previously Presented) A vaccine for inducing an immune response comprising an antigen conjugated to an anti-mouse Dendritic and Epithelial Cell-205 (DEC-205) antibody, wherein the antibody is reactive with the amino acid sequence as set forth in SEQ ID NO: 1.

37. (Previously Presented) The vaccine of any one of claims 26, 35, or 36, further comprising an immune stimulator.

38. (Previously Presented) The vaccine of claim 37, wherein the immune stimulator is selected from the group consisting of a cytokine, a lymphokine, and an adjuvant.

39. (Previously Presented) The vaccine of any one of claims 26, 35, or 36, wherein the antigen is selected from the group consisting of a virus, a bacterium, a parasite, and a tumor.

40. (New) A vaccine for inducing an immune response comprising an antigen conjugated to an antibody which binds mouse Dendritic and Epithelial Cell 205 (DEC-205) having the amino acid sequence of SEQ ID NO: 3 , wherein the antibody cross reacts with human DEC-205.

41. (New) The vaccine of claim 40, further comprising an immune stimulator.

42. (New) The vaccine of claim 41, wherein the immune stimulator is selected from the group consisting of a cytokine, a lymphokine, and an adjuvant.

43. (New) The vaccine of claim 40, wherein the antigen is selected from the group consisting of a virus, a bacterium, a parasite, and a tumor antigen.

44. (New) The method of claim 40, wherein the antigen is bound to the antibody to DEC-205 by means of a cross-linking agent.

45. (New) The method of claim 40, wherein a light chain or a heavy chain of the antibody to DEC-205, and the antigen, are present on a single polypeptide chain.

EVIDENCE APPENDIX

Appendix B is a copy of the Declaration by Dr. Michel Nussenzweig, originally submitted with the Amendment and Response filed by Appellants dated January 3, 2005.

Appendix C is a copy of Guo *et al.*, *Hum Immunol.* 2000 Aug; 61(8):729-38, which was cited in the Declaration by Dr. Michel Nussenzweig that was submitted with the Amendment and Response filed by Appellants on January 3, 2005.

RELATED PROCEEDINGS APPENDIX

Please note that a Pre-Appeal Brief Request for Review and related Appeal have been filed in the Continuation-in-Part application, U.S.S.N.: 09/925,284 (filed August 9, 2001). A Notice of Panel Decision from the Pre-Appeal Brief Review issued on August 8, 2006 and an Appeal Brief was filed on November 27, 2006 with a Petition for a three month extension of time.



600-1-081CONCIP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Steinman et al.

EXAMINER: Schwadron, Ronald B.

SERIAL NO.: 09/925,284

ART UNIT: 1644

FILED: August 9, 2001

FOR: ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE
IMMUNE RESPONSE THEREFROM

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, MICHEL NUSSENZWEIG, hereby declare and state that:

1. I am a Howard Hughes Investigator, Sherman Fairchild Professor and Senior Physician at Rockefeller University having received my Ph.D. degree from the Rockefeller University in 1981 and my M.D. degree from New York University in 1982. I received postdoctoral medical and scientific training at Harvard University. My full curriculum vitae is attached hereto as Exhibit A.

2. My principal area of research is in Immunology and among other positions I serve as reviewer in numerous funding agencies of many countries, including the National Institute of Health, March of Dimes, Dana Foundation. I also have served as reviewer for numerous scientific journals, and I am the Editor of the Journal of Experimental Medicine and the Journal of Immunologic Methods.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM", having U.S. Serial Number 09/925,284, which is a continuation-in-part of U.S. application Serial Number 09/586,704, filed on June 5, 2000, which is a continuation of U.S. Serial Number 08/381,528, filed on January 31, 1995, now abandoned.

BEST AVAILABLE COPY

4. I have reviewed the disclosure of the present application, with particular emphasis on the support in the application as filed for the preparation and generation of antibodies against human and mouse DEC-205 proteins.

5. The present application claims a method of enhancing the development of tolerance to a pre-selected antigen for which tolerance is desired in a mammal comprising exposing ex vivo or in vivo dendritic cells from said mammal to a conjugate comprising said pre-selected antigen covalently bound to an anti-human DEC-205 antibody or an anti-murine DEC-205 antibody that binds to human DEC-205 under conditions that promote dendritic cell quiescence. More particularly, the pre-selected antigen is selected from the group consisting of allergens, autoantigens and antigens participating in allograft rejection. The human DEC-205 protein has a carboxy terminal and amino terminal amino acid sequence as disclosed in the parent application, U.S. Serial Number 09/586,704, filed on June 5, 2000, as SEQ ID NOs.: 1 and 2, respectively. Furthermore, as noted in the parent application USSN 09/586,704, the first 19 amino acid residues of the amino terminal human DEC-205 protein (designated as SEQ ID NO: 13 in the parent application) were used to generate antibodies that reacted with human DEC-205. The sequences from the parent application have now been included in the sequence listing for the present application and are designated as SEQ ID NO: 7 for the carboxy terminal, SEQ ID NO: 8 for the amino terminal, and SEQ ID NO: 9 for the first 19 amino acids of the amino terminal used for antibody generation.

6. The subject matter of the present application was based on work performed in my laboratory, whereby the human DEC-205 molecule was cloned and expressed (Guo, M., Gong, S., Maric, S., Misulovin, Z., Paek, M., Mahnke, K., Nussenzweig, M.C. & Steinman, R.; (2000), A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells, *Human Immunology* 61:729-738). Anti-human DEC-205 antibodies were then prepared by immunizing animals with the first 19 amino acid residues from the N terminal fragment of the cloned human DEC-205 protein.

7. To summarize briefly, the cloning of human DEC-205 was done through use of a cDNA fragment of the 3' portion of mouse DEC-205. This was used to screen a human lymphocyte and thymus cDNA library using standard procedures known to those skilled in the art. In particular, the cDNA fragment of mouse DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5'-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were identified by DNA sequencing on

both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dyc determinator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371). The human cDNAs were expressed in pEF-BOS modified to carry a 3' human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and Fc domains were amplified from plasmids by PCR using 5' MG31 primers and 3' MG35 primers. The 5' -- primer contains a SpeI site, while the 3' -- primer contains a NotI site and codes for FcR at the junction point of DEC-205 and the Fc tag. The human DEC-205 Fc fusion protein was produced by transiently transfecting 293 cells using calcium phosphate mediated gene transfer. The fusion protein was purified on protein A sepharose and was then used to inject mice. Following several booster injections, the serum was tested for antibodies that reacted with the CR-Fc domain of the human DEC-205 molecule using Western blot procedures. Afterwards, the spleens were harvested from those animals showing a positive reaction and were fused with SP2/0 cells. The supernatants were screened by ELISA, dot blot, thymus tissue staining and FACS analysis. Cell clones that secreted anti-human DEC-205 antibodies were further subcloned and expanded.

8. The present application teaches methods for inducing tolerance by conjugating an antigen to a DEC-205 antibody for targeting to the DEC-205 receptor on specific cells, such as dendritic cells, under conditions that promote dendritic cell quiescence. The antibodies that react with the DEC-205 proteins, in particular, the anti-human DEC-205 antibodies, were prepared using the first 19 amino acid residues from the amino terminal end of the cloned human DEC-205 protein, as described in the parent application U.S. Serial Number 09/586,704, and further attested to in this declaration. Thus, it is my belief that the disclosure of the present application provides sufficient written description for a person skilled in the art to prepare such antibodies that react with human DEC-205 protein as presently claimed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: 1/3/05

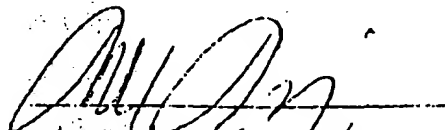

Michel Nussenzweig, M.D., Ph.D.

EXHIBIT A

CURRICULUM VITAE

Name: Michel C. Nussenzweig

Date of Birth: February 10, 1955

Education:

1975 B.A. - New York University College of Arts and Sciences
1981 Ph.D. - The Rockefeller University
1982 M.D. - New York University School of Medicine

Clinical Training:

1982-1985 Intern & Resident, Internal Medicine
Massachusetts General Hospital
1984-1985 Clinical Fellow, Infectious Diseases
Massachusetts General Hospital

Postdoctoral Training:

1986-1989 Harvard Medical School, Department of Genetics

Professional Appointments

1990-1996 Assistant & Associate Professor, The Rockefeller University
1990-1999 Assistant & Associate Investigator, Howard Hughes Medical Institute
1996-present Professor & Senior Physician, The Rockefeller University
1999-present Investigator, Howard Hughes Medical Institute
2000-present Sherman Fairchild Professor of Immunology, The Rockefeller Univ.

Honors & Awards

Summa Cum Laude, New York University College of Arts and Sciences - 1975; Phi Beta Kappa, New York University College of Arts and Sciences - 1975; Alpha Omega Alpha, New York University Medical School - 1982; Bertram M. Gresner Memorial Research Award, New York University School of Medicine - 1982; Elected Member American Society of Clinical Investigators - 1997, Solomon A. Berson Award for Basic Science - 2002

Teaching:

Immunology, Course Organizer

Institutional:

Chair, Transgenic Facility Coordinating Committee
Chair, Animal Care and Use Committee

Chair, Hospital Seminar Committee
Member, Immunology Search Committee
Member, Institutional Review Board for Biohazards, Radioisotopes, Toxic Chemicals, and Carcinogens
Member Hospital GCRC Scientific Advisory Committee
Elected Senior Faculty Representative Academic Council
Member, Virology Search Committee

National

Arthritis Foundation Molecular Immunology study section 1993-1996
NIH Immunobiology Study Section Ad Hoc reviewer 1998, and 1999
NIH ALY Study Section Ad Hoc Reviewer, 1999
NIH NIAID Council Ad Hoc 1998
Organizer Keystone Symposium on Dendritic Cells 1998
Organizer Keystone Symposium on B Cells 1999
March of Dimes Review Committee 1999-
External Reviewer LMGD NICHD 2000
Damon Runyon Cancer Research Fund Review Committee 2000-2002
American Association of Immunologists Program Committee 2000-
NIH ALY Study Section Member 2001-
Organizer Keystone Symposium on B Cell Biology 2003

Editorial:

1996-Present	Editor, The Journal of Experimental Medicine
1999-Present	Editor, The Journal of Immunological Methods
2000-Present	Transmitting Editor, International Immunology
2002-Present	Advisory Editor, Nature Reviews Immunology

Consultant:

Abgenix, Fremont, CA
Zycos, Lexington MA

Professional Memberships:

American Association of Immunologists
American Medical Association
The New York Academy of Sciences
Kunkel Society
Harvey Society

Publications:

1. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Chen, L.L. & Cohn, Z.A. Dendritic Cells: an important new cell type in the mixed leukocyte reaction. In: Kaplan JG, ed. Proc. of the 13th International Leukocyte Culture conference. *Elsevier/North Holland Publ. Co.* 273. (1979)
2. Steinman, R.M., & Nussenzweig, M.C. Dendritic cells features and functions. *Immunol. Rev.* 53:127-147. (1980)
3. Nussenzweig, M.C. & Steinman, R.M. Contribution of dendritic cells to stimulation of the syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196-1212. (1980)
4. Nussenzweig, M.C., Steinman, R., Gutchinov, B., & Cohn, Z.A. Dendritic cells are accessory cell for the development of anti-trinitrophenyl cytotoxic T cells. *J. Exp. Med.* 152:1070-1084. (1980)
5. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Chen, L.L., Schlesinger, S.J., & Cohn, Z.A. Dendritic Cells of the Mouse Identification and Characterization. *J. Invest. Dermatol.* 75:14-16. (1980)
6. Nussenzweig, M.C., Steinman, R.M., Unkeless, J.C., Witmer, M., Gutchinov, B., & Cohn, Z.A. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J. Exp. Med.* 154:168-187. (1981)
7. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Gutchinov, B., & Austyn, J.M. Studies with a monoclonal antibody to mouse dendritic cell. *Transplantation* 31:151. (1981)
8. Nussenzweig, M.C., & Steinman, R.M. Surface of dendritic cells. *Immunology Today* 3:65 (1982)
9. Nussenzweig, M.C., Steinman, R.M., Witmer, M.D., & Gutchinov, B. A monoclonal antibody specific for mouse dendritic cells. *Pro. Natl. Acad. Sci., USA* 79:161-165. (1982)
10. Steinman, R.M., Gutchinov, B., Witmer, M.D., & Nussenzweig, M.C. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613-627. (1983)
11. Nussenzweig, M.C., Shaw, A.C., Sinn, E., Danner, D.B., Holmes, K.L., Morse, H.C., & Leder, P. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin. *Science* 236:816-819. (1987)

12. Nussenzweig, M.C., Shaw, A.C., Sinn, E., Torres, J., & Leder, P. Allelic exclusion in transgenic mice that carry human mutant human immunoglobulin genes. *J. Exp. Med.* 167:1969-1974 (1988)
13. Nussenzweig, M.C., Schmidt, E.V., Shaw, A.C., Sinn, E., Torres, J., Mathey-Prevot, B., Pattengale, P.K. & Leder, P. A human immunoglobulin gene reduces the incidence of lymphomas in c-Myc bearing transgenic mice. *Nature* 336:446-450. (1989)
14. Morton, C.C., Nussenzweig, M.C., Sousa, R., Sorenson, G.D., Pettengill, O.S. & Shows, T.B. Mapping and characterization of an X-linked processed gene related to mycl. *Genomics* 4:367-375. (1989)
15. Shimizu, A., Nussenzweig, M.C., Tataunobu-Ryushin, M., Leder, P., & Honjo, T. Immunoglobulin double isotype expression by trans-mRNA in a human immunoglobulin transgenic mouse. *Proc. Natl. Acad. Sci. USA.* 86:8020-8023. (1989)
16. Shimizu, A., Kinashi, T., Nussenzweig, M.C., Tataunobu-Ryushin, M., Leder, P., & Honjo, T. Molecular mechanism for immuno-globulin double-isotope expression. *Cold Spring Harbor Symposia on Quantitative Biology* Vol. LIV 175.
17. Friedlander, R., Nussenzweig, M.C., & Leder, P. Complete nucleotide sequence of the membrane form of the human IgM heavy chain. *Nucleic Acids Res.* Vol. 18(14):4278. (1990)
18. Shimizu, A., Nussenzweig, M.C., Han, H., Sanchez, M., & Honjo, T. Trans-splicing as a Possible Molecular Mechanisms for the Multiple Isotope Expression of the Immunoglobulin Gene. *J. Exp. Med.* 173:1385-1393. (1991)
19. Costa, T., Suh, H-Y., & Nussenzweig, M.C. Chromosomal Position of Rearranging Gene Segments Influences Allelic Exclusion in Transgenic Mice. *Proc. Natl. Acad. Sci. USA* 89:2205-2208. (1992)
20. Costa, T., Franke, R.R., Sanchez, M., Misulovin, Z., & Nussenzweig, M.C. Functional Reconstitution of an Immunoglobulin Antigen Receptor in T Cells. *J. Exp. Med.* 175:1669-1676. (1992)
21. Sanchez, M., Misulovin, Z., Burkhardt, A.L., Mahajan, S., Costa, T., Franke, R.R., Bolen, J.B. & Nussenzweig, M.C. Signal Transduction by Immunoglobulin Is Mediated Through Ig α and Ig β . *J. Exp. Med.* 178:1049-1055 (1993)
22. Burkhardt, A.L., Costa, T., Misulovin, Z., Stealy, B., Bolen, J.B., & Nussenzweig, M.C. Ig α and Ig β are Functionally Homologous to the Signaling Protein of the T cell Receptor. *Molecular & Cellular Biology* Vol. 14(2):1095-1103. (1994)

23. Kurosaki, T., Muta, T., Sanchez, M., Misulovin, Z., **Nussenzweig, M.C.**, & Ravetch, J.V. A 13 amino acid motif in the cytoplasmic domain of FcγRIIB modulates B cell receptor signalling. *Nature* Vol. 368:70-73. (1994)
24. Spanopoulou, E., Roman, C.A.J., Corcoran, L., Schlissel, M.S., Silver, D.P., Storb, U., Nemazee, D., **Nussenzweig, M.C.**, Shinton, S.A., Hardy, R.R., & Baltimore, D. Expression of functional immunoglobulin transgenes allows ordered B-cell differentiation to progress in Rag-1 deficient mice. *Genes & Development* Vol. 8:1030-1042. (1994)
25. Wayne, J., Suh, H., Misulovin, Z., Sokol, K.A., Inaba, K., & **Nussenzweig, M.C.** A Regulatory Role for Recombinase Activating Genes, RAG-1 and RAG-2, in T Cell Development. *Immunity* Vol. 1:95-107. (1994)
26. Qin, X.F., Luo, Y., Suh, H.-Y., Wayne, J., Misulovin, Z., Gärster, T., Roeder, R.G. & **Nussenzweig, M.C.** Transformation by homeobox genes can be mediated by selective transcriptional repression. *EMBO J.* Vol. 13:5967-5976. (1994)
27. Wayne, J., Suh, H.-Y., Sokol, K., Petrie, H.T., Witmer-Pack, M., Edelhoff, S., Disteche, C.M., & **Nussenzweig, M.C.** TRC Selection and Allelic Exclusion in RAG Transgenic Mice that Exhibit Abnormal T Cell Localization in Lymph Nodes and Lymphatics. *J. Immunol.* Vol. 153:5491-5502. (1994)
28. Mitchell, R.N., Barnes, K.A., Grupp, S.A., Sanchez, M., Misulovin, Z., **Nussenzweig, M.C.** & Abbas, A.K. Intracellular Targeting of Antigens by Membrane Immunoglobulin in B Lymphocytes *J. Exp. Med.* 181:1705-1714. (1995)
29. Heufler, C., Humborg, C., Steinman, R.M., Schuler, G. and **Nussenzweig, M.C.** A molecular strategy to identify molecules that are specific for dendritic cells and/or critical to their unique immunostimulatory function. *Adv. Exp. Med Biol.* 378:367-369 (1995)
30. Papavasiliou, F., Misulovin, Z., Suh, H.-Y., **Nussenzweig, M.C.** The role of Igβ in Precursor B cell Transition and Allelic Exclusion. *Science* Vol. 268:408-411 (1995)
31. Jiang, W., Swiggard, W., Heufler, C., Peng, M., Steinman, R.M., & **Nussenzweig, M.C.** The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 375:151-155. (1995)
32. Inaba, K., Swiggard, W.J., Inaba, M., Meltzer, J., Mirza, Sasagawa, T., **Nussenzweig, M.C.**, & Steinman, R.M. Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse Leukocytes. *Cell. Immunol.* 163:148-156 (1995)
33. Swiggard, W.J., Asra, M., **Nussenzweig, M.C.**, & Steinman, R.M. DEC-205, a 205 kDa Protein abundant on mouse dendritic cells and thymic epithelium that is detected by the

- monoclonal antibody NLDC-145: Purification, characterization and N-terminal amino acid sequence. *Cell. Immunol.* 165:302-311 (1995)
34. Papavasiliou, F., Jankovic, M., Suh, H.-Y., Nussenzweig, M.C. The Cytoplasmic Domains of Ig α and Ig β can Independently Induce the Precursor B cell Transition and Allelic Exclusion. *J. Exp. Med.* 182(5):1389. (1995)
35. Gong, S., & Nussenzweig, M.C. Regulation of an Early Developmental Checkpoint in the B cell Pathway by Ig β . *Science* 272:411-414 (1996)
36. Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., Nussenzweig, M.C., & Li, G.C. Requirement for Ku80 in Growth and V(D)J Recombination. *Nature* 382:551-555 (1996)
37. Kim, U., Qin, X., Gong, S., Stevens, S., Luo, Y., Nussenzweig, M.C., & Roeder, R.G. The B cell specific transcription coactivator OCA-B is essential for normal production of immunoglobulin isotypes. *Nature* 383:542-547 (1996)
38. Papavasiliou, F., Jankovic, M., & Nussenzweig, M.C. Surrogate or Conventional Light Chains Are Required for Membrane Immunoglobulin Mu to Activate the Precursor B Cell Transition. *J. Exp. Med.* 184:2025-2030 (1996)
39. Gong, S., Sanchez, M. & Nussenzweig, M.C. Counterselection against Du Is Mediated through Immunoglobulin (Ig) α -Ig β . *J. Exp. Med.* 184:2079-2084 (1996)
40. Cortes, P., Weis-Garcia, F., Misulovin, Z., Nussenzweig, A., Lai, J.S., Li, G., Nussenzweig, M.C., & Baltimore, D. In vitro V(D)J recombination: signal joint formation. *Proc. Natl. Acad. Sci. USA* 93: 14003-13 (1996)
41. Sawchuk, D.J. Weis-Garcia, F. Malik, S., Besmer, E. Bustin, M. Nussenzweig, M.C. & Cortes, P. V(D)J recombination: Modulation of RAG1 and RAG2 cleavage activity on 12/23 substrates by whole cell extract and DNA bending proteins. *J. Exp. Med.* 185:2025-2032 (1997)
42. Weis-Garcia, F., Besmer, E. Yu, W., Sawchuk, D., Hu, Y., Nussenzweig, M.C. & Cortes, P. V(D)J recombination: In vitro coding joint formation. *MCB* 17:6379-6385(1997)
43. Papavasiliou, F., Jankovic, M., Gong, S. & Nussenzweig, M.C. Control of immunoglobulin gene rearrangements in developing B cells, review. *Current Opinions in Immun.* 9:233-244 (1997)
44. Nussenzweig, M.C. Tails to teach a B cell. *Current Biology.* (1997)

45. Nussenzweig, M.C. & Allison, Jim. Lymphocyte activation and effector functions. How signals are integrated in the immune system (Editorial Overview) *Current Opinions in Immun.* 9:293-295 (1997)
46. Papavasiliou, F., Casellas, R., Suh, H., Qin, X., Besmer, E., Pelanda, R., Nemazee, D., Rajewsky, K., and Nussenzweig, M.C. V(D)J Recombination in Mature B cells: a Mechanism for Altering Antibody Responses. *Science* 278:298-301 (1997)
47. Honghai, O., Nussenzweig, A., Kurimasa, A., Soares, V., Li, X., Cordon-Cardo, C., Li, W., Cheong, N., Nussenzweig, M.C., Iliakis, G., Chen, D.J. & Li, G.C. Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination in vivo. *J. Exp. Med.* 186:921-929 (1997)
48. Lee, S.Y., Reichlin, A., Santana, A., Sokol, K.A., Nussenzweig, M.C. & Choi, Y. TRAF2 is essential for JNK but not NF- κ B activation and regulates lymphocyte proliferation and survival. *Immunity* 7:703-713 (1997)
49. Casellas, R., Nussenzweig, A., Wuerffel, R., Pelanda, R., Reichlin, A., Suh, H., Qin, X-F., Besmer, E., Kenter, A., Rajewsky, K. and Nussenzweig, M.C. Ku80 is required for immunoglobulin isotype switching. *EMBO Vol* 17 8:2404-2411 (1998)
50. Meffre, E., Papavasiliou, F., Cohen, P., de Bouteiller, O., Bell, D., Karasuyama, H., Schiff, C., Banchereau, J., Liu, Y-J., and Nussenzweig, M.C. Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B cells. *J. Exp. Med.* 188:765-772 (1998)
51. Qin, X-F., Reichlin, A., Luo, Y., Roeder, R.G. and Nussenzweig, M.C. OCA-B integrates B cell antigen receptor-, CD40L-, and-IL-4-mediated signals for the germinal center pathway of B cell development. *EMBO* 17:5066-5075 (1998)
52. Besmer, E., Mansilla-Soto, J., Cassard, S., Sawchuk, D., Brown, G., Sadofsky, M., Lewis, S., Nussenzweig, M.C. and Cortes, P. Hairpin coding end opening by the recombination activating genes RAG1 and RAG2. *Molecular Cell* 2:817-828 (1998)
53. Qin, X-F., Schwers, S., Yu, W., Papavasiliou, F., Suh, H., Nussenzweig, A., Rajewsky, K., and Nussenzweig, M.C. Secondary V(D)J recombination in B-1 cells. *Nature* 397:355-359 (1999)
54. Nussenzweig, M.C. Immune Receptor Editing: Revise and Select. *Cell* 95:875-878 (1998)
55. Yu, W., Nagaoka, H., Jankovic, M., Misulovin, Z., Suh, H., Rolink, A., Melchers, F., Meffre, E., & Nussenzweig, M.C. Continued RAG expression in late stages of B cell development and no apparent re-induction after immunization. *Nature* 400:682-687 (1999)

56. Yu, W., Misulovin, Z., Suh, H., Hardy, R.R., Jankovic, M., Yannoutsos, N & Nussenzweig, M.C. Coordinate regulation of RAG1 and RAG2 by cell type-specific DNA elements 5' of RAG2. *Science* 285:1080-1084 (1999)
57. Tiefenthaler, M., Marksteiner, R., Neyer, S., Koch, F., Hofer, S., Schuler, G., Nussenzweig, M.C., Schneider, R., & Heufler, C. M1204, a Novel 2',5' Oligoadenylate Synthetase with a Ubiquitin-Like Extension, Is Induced During Maturation of Murine Dendritic Cells^{1,2}. *Journal of Immun.* 163:760-765 (1999)
58. Santagata, S., Besmer, E., Villa, A., Bozzi, F., Allingham, J.S., Sobacchi, C., Haniford, D.B., Vezzoni, P., Nussenzweig, M.C., Pan, Z-Q., & Cortes, P. The RAG1/RAG2 complex a 3' flap endonuclease: implications for junctional diversity in V(D)J and transpositional recombination. *Molecular Cell* 4:935-947 (1999)
59. Yu, W., Nagaoka, H., Misulovin, Z., Meffre, E., Suh, H., Jankovic, M., Yannoutsos, N., Casellas, R., Besmer, E., Papavasiliou, F., Qin, X., & Nussenzweig, M.C. RAG Expression in B Cells in Secondary Lymphoid Tissues. Cold Spring Harbor Symposia on Quantitative Biology Vol. LXIV:207-210, (1999)
60. Nagaoka, H., Yu, W. & Nussenzweig, M.C. Regulation of RAG expression in developing lymphocytes. *Current Opinion in Immunology* 12:187-190 (2000)
61. Kronin, V., Wu, L., Gong, S., Nussenzweig, M.C., & Shortman, K. DEC-205 as a marker of dendritic cells with regulatory effects on CD8 T cell responses. *International Immunology* 12(5):731-735 (2000)
62. Liu, Y., Chirino, A.J., Misulovin, Z., Leteux, C., Feizi, T., Nussenzweig, M.C., & Bjorkman, P.J. Crystal structure of the cysteine-rich domain of mannose receptor complexed with a sulfated carbohydrate ligand. *J. Exp. Med.* 191(7):1105-1115 (2000)
63. Leteux, C., Chai, W., Loveless, R.W., Yuen, C-T., Uhlin-Hansen, L., Combarnous, Y., Jankovic, M., Maric, S.C., Misulovin, Z., Nussenzweig, M.C. & Feizi, T. The cysteine-rich domain of the macrophage mannose receptor is a multispecific lectin that recognizes chondroitin sulfates A and B and sulfated oligosaccharides of blood group Lewis^a and Lewis^x types in addition to the sulfated N-glycans of lutropin. *J. Exp. Med.* 191(7):1117-1126 (2000)
64. Difilippantonio, M.J., Zhu, J., Chen, H.T., Meffre, E., Nussenzweig, M.C., Max, E.E., Ried, T. & Nussenzweig, A. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404:510-514 (2000)
65. Nagaoka, H., Gonzalez-Aseguinolaza, G., Tsuji, M. & Nussenzweig, M.C.

Immunization and infection change the number of recombination activating gene (RAG)-expressing B cells in the periphery by altering immature lymphocyte production. *J. Exp. Med.* 191(12):2113-2120 (2000)

66. Mahnke, K., Guo, M., Lee, S., Sepulveda, H., Swain, S.L., **Nussenzweig, M.C.** & Steinman, R.M. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via MHC II+, lysosomal compartments. *J. of Cell Biology* 151:673-683 (2000)
67. Meffre, E., Davis, E., Schiff, C., Cunningham-Rundles, C., Ivashkiv, L.B. Staudt, L.M., Young, J.W. & **Nussenzweig, M.C.** Circulating human B cells that express surrogate light chains and edited receptors. *Nature Immunology* 1(3):207-213 (2000)
68. Guo, M., Gong, S., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., **Nussenzweig, M.C.** & Steinman, R. A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Human Immunology* 61:729-738 (2000)
69. Reichlin, A., Hu, Y., Meffre, E., Nagaoka, H., Gong, S., Kraus, M., Rajewsky, K., & **Nussenzweig, M.C.** B cell development is arrested at the immature B cell stage in mice carrying a mutation in the cytoplasmic domain of Ig β . *J. Exp. Med.* 193(1):13-23 (2001)
70. Kraus, M., Pao, L.I., Reichlin, A., Hu, Y., Canono, B., Cambier, J.C. **Nussenzweig, M.C.** & Rajewsky, K. Interference with Ig α ITAM phosphorylation modulates or blocks B cell development, depending on the availability of an Ig β cytoplasmic tail. *J. Exp. Med.* 194(4):455-470 (2001)
71. Meffre, E., Casellas, R., **Nussenzweig, M.C.** Antibody regulation of B cell development. *Nature Immunology* 1(5):379-385 (2000)
72. Casellas, R., Yang Shih, T-A., Kleinewietfeld, M., Rakonjac, J., Nemazee, D., Rajewsky, K. & **Nussenzweig, M.C.** Contribution of receptor editing to the antibody repertoire. *Science* 291:1541-1544 (2001)
73. Wurbel, M-A., Malissen, M., Guy-Grand, D., Meffre, E., **Nussenzweig, M.C.**, Richelme, M., Carrier, A., and Malissen, B. Mice lacking the CCR9 -chemokine receptor show a mildly impeded of early T and B cells and a reduction in TCR⁺ gut intraepithelial lymphocytes. *Blood* 98(9):2626-2632 (2001)
74. Meffre, E., Chiorazzi, M. and **Nussenzweig, M.C.** Circulating human B cells that express surrogate light chains display a unique antibody repertoire. *J. Immunol.* 167(4):2151-2156 (2001)
75. Meffre, E. Catalan, N., Seltz, F., Fischer, A., **Nussenzweig, M.C.*** and Durandy, A. Somatic hypermutation shapes the antibody repertoire of memory B cells in humans. *J. Exp. Med.* 194(3):375-378 (2001)

*Equal contributing senior author

76. Yannoutsos, N., Wilson, P., Yu, W., Chen, H.T., Nussenzweig, A., Petri H. and **Nussenzweig, M.C.** The role of recombination activating gene (RAG) reinduction in thymocyte development in vivo. *J. Exp. Med.* 194(4):471-480 (2001)
77. Meffre, E., Milili, M., Blanco-Betancourt, C., Antunes, H., **Nussenzweig, M.C.*** and Schiff, C. Immunoglobulin heavy chain expression shapes the B cell receptor repertoire in human B cell development. *J. Clin. Invest.* 108(6):879-886 (2001)

*Equal contributing senior author

78. Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M. and **Nussenzweig, M.C.** Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med.* 194(6):769-779 (2001)
79. Petersen, S., Casellas, R., Reina San-Martin, B., Chen, H.T., Difilippantonio, M.J., Wilson, P.C., Hanitsch, L., Celeste, A., Muramatsu, M., Pilch, D.R., Redon, C., Ried, T., Bonner, W.M., Honjo, T., **Nussenzweig, M.C.**, and Nussenzweig, A. AID is required to initiate Nbs1/γ-H2AX focus formation and mutations at sites of class switching. *Nature* 414:660-665. (2001)
80. Steinman, R.M. and **Nussenzweig, M.C.** Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci.* 99(1):351-358 (2002)
81. Pelandra, R., Braun, U., Hobeika, E., **Nussenzweig, M.C.** and Reth, M. Ig-α and Ig-β are not required for the development of B cell progenitor cells and the rearrangement of IgH chain genes. *J. of Immunology* 169(2):865-872 (2002)
82. Lee, S.J., Evers, S., Roeder, D., Parlow, A.F., Risteli, J., Risteli, L., Lee, Y.C., Feizi, T., Langen, H. and **Nussenzweig, M.C.** Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science* 295(5561):1898-901 (2002)
83. Shih, T.A., Roederer, M. and **Nussenzweig, M.C.** Role of antigen receptor affinity in T cell-independent antibody responses in vivo. *Nat Immunol* 3(4):399-406 (2002)
84. Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola V., Meffre, E., Difilippantonio, M.J., Redon, C., Pilch, D.R., Oлару, A., Eckhaus, M., Camerini-Otero, R.D., Tessarollo, L., Livak, F., Manova, K., Bonner, W.M., **Nussenzweig, M.C.**, Nussenzweig, A. Genomic Instability in Mice Lacking Histone H2AX. *Science* 296:922-927 (2002)

85. Shih, T. A., Meffre, E., Roederer, M. and Nussenzweig M.C. Role of BCR affinity in T cell dependent antibody responses *in vivo*. *Nat. Immunol.* 3(6):570-575 (2002)
86. Guy-Grand, D., Azogui, O., Celli, S., Darche, S., Nussenzweig, M.C., Kourilsky, P., and Vassalli, P. Extrathymic lymphopoiesis: ontogeny and contribution to gut intraepithelial lymphocytes in athymic and euthymic mice. *J. Exp. Med.* 197(3):333-341 (2003)
87. Lee, S.J, Gonzalez-Aseguinolaza, G. and Nussenzweig, M.C. Disseminated Candidiasis and Hepatic Malarial Infection in Mannose-Binding Lectin-A Deficient Mice. *Mol. & Cellular Biology* Vol. 22:8199-8203 (2002)
88. Casellas, R., Jankovic, M., Meyer, G., Gazumyan, A., Luo, Y., Roeder, R.G., and Nussenzweig, M.C. OcaB is Required for Normal Transcription and V(D)J Recombination of a Subset of Immunoglobulin κ Genes. *Cell* Vol. 110:575-585 (2002)
89. Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M. C., and Steinman, R.M. Efficient Targeting of Protein Antigen to the Dendritic Cell Receptor DEC-205 in The Steady State Leads to Antigen Presentation on Major Histocompatibility Complex Class I Products and Peripheral CD8(+) T Cell Tolerance. *J Exp Med.* 196(12):1627-1638. (2002)
90. Meffre E, Nussenzweig, M.C. Deletion of immunoglobulin beta in developing B cells leads to cell death. *Proc Natl Acad Sci USA* 99(17):11334-11339 (2002)
91. Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21:685-711. (2003)
92. Lee S.J., Zheng, N-Y, Clavijo M, Nussenzweig M.C. Normal host defense during systemic candidiasis in mannose receptor-deficient mice. *Infect Immun.* 71(1):437-45. (2003)
93. Ramiro A.R., Stavropoulos P., Jankovic M, Nussenzweig M.C. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. *Nat Immunol.* 4(5):452-456 (2003)
94. Reina-San-Martin, B., Difilippantonio, S., Hanitsch, L., Masilamani, R., Nussenzweig, A. and Nussenzweig, M.C. γ -H2AX is required for recombination between immunoglobulin switch regions but not for intra-switch region recombination or somatic hypermutation. *J Exp Med.* 197(12):1767-1778 (2003)
95. Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., and Nussenzweig, M.C. Predominant autoantibody production by early human B cell precursor. *Science* 301:1374-1377 (2003)

A Monoclonal Antibody to the DEC-205 Endocytosis Receptor on Human Dendritic Cells

Ming Guo, Schiaoching Gong, Svetlana Maric,
Ziva Misulovin, Maggi Pack, Karsten Mahnke,
Michel C. Nussenzweig, and Ralph M. Steinman

ABSTRACT: DEC-205 is a multilectin receptor for adsorptive endocytosis, expressed in mouse dendritic cells (DC) and some epithelia. DEC-205 is homologous to the macrophage mannose receptor (MMR). A cDNA for murine DEC-205 was used to identify 3 overlapping human DEC-205 clones from a lymphocyte library. The human homologue is a transmembrane protein of 1722 aminoacids with 10 externally disposed C-type lectin domains having 77% identity to the mouse counterpart. The NH₂ terminal cysteine-rich and fibronectin type II domains were expressed and used to immunize mice. A hybridoma, MG38, which specifically recognized the immunogen was obtained from a DEC-205 knockout mouse. The antibody precipitated a 205 kD protein from metabolically labeled, monocyte-derived DCs. MG38 labeled mature monocyte-derived DCs but showed weak or no labeling of other

peripheral blood mononuclear cells. In tissue sections, MG38 identified DEC-205 on thymic cortical epithelium and DCs in the thymic medulla and tonsillar T cell areas. In contrast, an anti-MMR antibody stained DEC-205 negative, macrophages in the thymus cortex, the trabeculae of the thymus and tonsil, as well as efferent lymphatics in the tonsil. Therefore, the MG38 anti-DEC-205 antibody is useful for identifying DCs and reveals clear differences in sites where MMR and DEC-205 are expressed in lymphoid tissues. *Human Immunology* 61, 729–738 (2000). © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: dendritic cells; antigen receptor; C-type lectin; DEC-205; monoclonal antibody

ABBREVIATIONS

MMR macrophage mannose receptor

DC dendritic cell

INTRODUCTION

Several receptors for adsorptive endocytosis and antigen presentation have now been identified on dendritic cells (DCs). These include Fcγ [1] and Fcε [2] receptors and two multilectin receptors, the macrophage mannose

receptor (MMR) [3] and DEC-205 [1]. The carbohydrate recognition domains of the MMR show specificity for mannose [3], and the MMR internalizes and presents mannosylated proteins [3, 4]. The ligands for DEC-205 are not yet known, but antibodies to DEC-205 are presented efficiently to Ig-reactive T cells [1]. Both the MMR and DEC-205 contain homologous amino terminal cysteine rich (CR) and fibronectin II domains followed by 8 and 10 contiguous C-type lectin domains respectively. Both molecules contain a single transmembrane domain and a cytosolic region with a coated pit localization sequence for adsorptive uptake.

DEC-205 is expressed by many different sources of mouse DCs [5–7], according to data with a rat-anti-mouse monoclonal antibody, originally termed NLDC-145 [5]. This monoclonal was used to clone DEC-205

From the Laboratory of Molecular Immunology (M.G., S.G., S.M., Z.M., M.P., K.M., M.C.N., R.M.S.), and Laboratory of Cellular Physiology and Immunology (M.G., S.G., S.M., Z.M., K.M.), and Howard Hughes Institute (M.C.N.), Rockefeller University, New York, New York, USA.

Address reprint requests to: Dr. Ralph M. Steinman, Rockefeller University, 1230 York Ave, New York, NY, 10021-6399, USA; Tel: (212) 327-8106; Fax: (212) 327-8875; E-Mail: steinma@rockvax.rockefeller.edu.

Received January 3, 2000; revised May 5, 2000; accepted May 9, 2000.

Supported by grants from the NIH, AI 39672, and the Human Science Frontiers program.

Human Immunology 61, 729–738 (2000)

© American Society for Histocompatibility and Immunogenetics, 2000
Published by Elsevier Science Inc.

0198-8859/00/\$—see front matter
PII S0198-8859(00)00144-0

and provide the first information on its function [1]. A monoclonal to mouse MMR has yet to be reported. In contrast, the human MMR is a potent immunogen in mice, and the corresponding monoclonals have revealed abundant expression of the MMR on monocyte-derived DCs [3]. No antibody to human DEC-205 is available, although HuDEC-205 has been cloned independently in our laboratory and by Kato *et al.* [8].

Here, we set out to prepare monoclonal antibodies to sequences from the predicted protein but only succeeded when we immunized DEC-205 knockout mice. We describe a monoclonal MG38 to huDEC-205. Expression of human DEC-205 is prominent in DCs rather than other cells in human blood. Using monoclonals to MMR and DEC-205, we find that these receptors are expressed in different sites in tissue sections of thymus and tonsil.

MATERIALS AND METHODS

Human DEC-205 cDNA

A cDNA fragment of the 3' portion of murine DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5'-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were characterized by DNA sequencing on both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dye terminator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371).

Expression of CR-FnII Domains of hDEC-205 in a Mammalian 293T Cell Expression System

cDNAs were expressed in pEF-BOS [9] modified to carry a 3' human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and FnII domains were amplified from plasmids by PCR using 5'-primers MG31: 5'-CGGGATCCACTAGTCGCGTGC GCCGAGG-3', and 3'-primer MG35: 5'-CTTAAAGCCTGAAAACG GTCCGCGCCGGGCGGCCGCATTCTTAT-3'. The 5'-primer contains a SpeI site, while the 3'-primer contains a Not I site and codes for PRR at the junction point of DEC-205 and the Fc tag (Fig. 1A). The hDEC-205-Fc fusion protein was produced by transiently transfecting 293T cells using calcium phosphate-mediated gene transfer. Medium containing the secreted recombinant protein was harvested after 10–12 days, and the recombinant protein was recovered by protein A chromatography [10].

Proteolysis and Antigen Purification

The CR-FnII portion of the fusion protein was separated from the Fc tag by papain digestion. Fusion protein

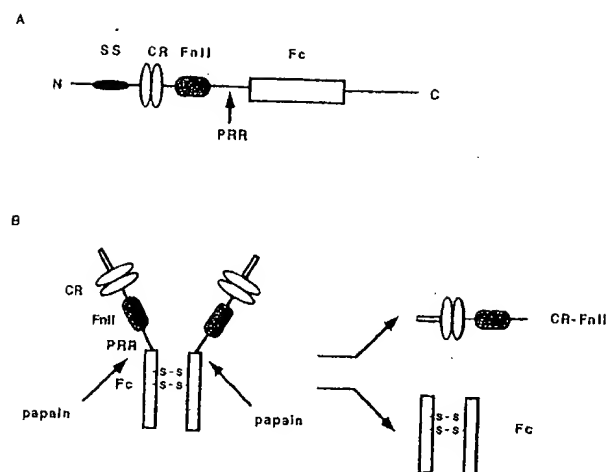


FIGURE 1 (A) Schematic of the CR-FnII-Fc fusion protein expression construct. SS: signal sequence; CR: cysteine-rich domain; FnII: fibronectin type II domain; Fc: human IgG Fc domain; PRR: three amino acids, proline-arginine-arginine, inserted at the junction point of the DEC domain and Fc domain. (B) Schematic of fragments produced by papain digestion. The secreted fusion protein forms dimers through S-S bonds in the Fc domain. Papain cleaves at the end of the Fc domain close to the junction point.

bound to Protein A Sepharose was digested with papain (Sigma Chemical Co., St. Louis, MO, USA) at a ratio of enzyme:protein of 1:100 in 1× PBS containing 10 mM EDTA and 10 mM cysteine for 30 min at 37°C (Fig. 1B). The reaction was terminated by adding iodoacetamide to a final concentration of 30 mM. Protein A beads and the bound Fc fragments were removed by centrifugation.

Immunization of DEC-205 Knockout Mice and Hybridoma Production

Purified CR-FnII protein in CFA (prime) or IFA (boost) were injected intraperitoneally four times into DEC-205 knock out (DEC-205^{-/-}) mice at 50 µg per inoculation. The DEC-205^{-/-} mice were produced by deleting the DEC-205 promoter and first coding exon in ES cells as previously performed for Igβ [11]. Initial immunophenotyping showed apparently normal B and T cell development in these mice, as well as T cell dependent immune responses. Sera were harvested from immunized mice and tested for the presence of anti-CR-FnII by western blot. Mice producing antibodies were reboosted with CR-FnII protein in PBS, their spleens were harvested 4 days later, and the splenocytes were fused with SP2/0 cells [10]. Supernatants were screened by ELISA, dot blot, thymus tissue section staining, and FACS analysis.

ELISA

Plates were coated with purified CR-FnII protein at a concentration of 2 µg/ml at 37°C for 2 hrs and blocked

30 min at room temperature with blocking buffer (0.25% BSA in PBS). Fifty μ l of supernatant from the growing fusion well was added and incubated at room temperature for 1 h, then washed. HRP-conjugated secondary antibody (1:5,000 dilution in blocking buffer) was added and incubated for 45–60 min at room temperature, then washed. Samples were developed using HRP substrate reagent (Bio-Rad Laboratories, Hercules, CA, USA).

Dot Blot

Ten to one hundred nanograms of antigen was loaded directly on the nitrocellulose paper. The nitrocellulose paper was blocked for 30 min at room temperature with 5% dry milk in wash buffer (PBS with 0.05% Tween 20), then incubated with hybridoma supernatants for 60 min at room temperature, and washed with washing buffer. HRP-conjugated-goat anti-mouse antibody (1:10,000 dilution in washing buffer) was added and incubated for 45 min at room temperature, then washed. The blots were developed with ECL detection reagent (NEN Life Science Products, Inc., Boston, MA, USA).

Preparation of Cells

PBMCs, monocytes, T cells, DCs, and macrophages were prepared from the blood of normal donors as described [12]. After isolation by sedimentation in Ficoll-Hypaque, PBMC were cultured in RPMI-5% human serum for 1 h. Floating cells were removed, and adherent cells were incubated 6 days in RPMI-5% human serum with GM-CSF (100 IU/ml, Leukine, Immunex) and IL-4 (1000 U/ml, Genzyme). Cells were fed on days 2, 4, and 6 with the same medium. Nonattached cells were harvested on day 6 as immature DCs, and to make mature DCs, the cells were cultured 3 more days in the above medium, cytokines and LPS. Macrophages were also obtained from PBMC by culture in parallel but in the absence of cytokines.

Immunohistochemistry

Frozen sections were cut at 10 μ from surgical specimens of human tonsil and thymus, generously provided by Dr. W.A. Muller (Cornell University Medical College, New York, NY, USA). Sections were cut onto glass slides (Carlson, Peotone, IL, USA), air dried, fixed in acetone and stained as follows. Biotinylated monoclonal antibodies, MG38 anti-DEC-205 and 3.29 anti-MMR [3] (a gift of Dr. A. Lanzavecchia, Basel), were applied at 20 and 5 μ g/ml, respectively. Alkaline phosphatase ABC (Vector) was applied for 30 min and the enzyme reaction developed using a BCIP/NBT substrate kit (Vector, Burlingame, CA, USA) according to manufacturer's instructions. Mouse serum (10%) was applied for 15 min, followed by leu 2 anti-CD8 hybridoma culture superna-

tant overnight and POX-anti mouse Ig (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h. Stable DAB (Research Genetics, Huntsville, AL, USA) was used for 6 min to develop the HRP reaction. The slides were mounted in PBS/glycerol. Alternatively, for two color immunofluorescence, thymic sections were labeled with the following sequence: 3.29 MMR at 2 μ g/ml followed by FITC-goat anti-human IgG1, then MG38 anti-DEC-205 at 2 μ g/ml followed by biotin goat anti-human IgG2b and Cy3 Streptavidin (all secondary antibodies from PharMingen). Stacks of 0.5 μ optical sections were taken by an Olympus epifluorescence microscope equipped with a motorized stage, a cooled CCD camera (Hamamatsu, Japan), and Metamorph software (Universal Imaging). Images were deconvoluted by applying a nearest neighbor algorithm provided by Metamorph.

Flow Cytometry

Fresh or permeabilized cells were incubated with hybridoma culture supernatant for 30 min on ice, washed with PBS-1% fetal calf serum, incubated with secondary antibody for 30 min on ice, then washed. Immunoglobulins were used at 1 μ g/ml and mAb-containing supernatant was used at 1:1 dilution. The secondary antibody was fluorescein isothiocyanate (FITC)-goat-anti-mouse immunoglobulin (1:200 dilution, Jackson). PE-CD14 (Becton Dickinson, Mansfield, MA, USA), PE-HLA-DR (Becton Dickinson), PE-CD83 (Immunotech) were used for double staining. The antibody to DC-LAMP [13] was provided by Drs. S. Lebecque and S. Saeland of the Laboratory for Immunology Research, Dardilly France. Stained cells were analyzed using FACScan flow cytometer (Becton Dickinson). For permeabilization, cells were fixed in 4% paraformaldehyde (Fisher Scientific Co., Pittsburgh, PA, USA) freshly made in PBS for 30 min on ice, followed by two washes with PBS. Fixed cells were permeabilized with 0.5% saponin (Sigma Chemical Co., St. Louis, MO, USA) in PBS with 1% FCS for 30 min on ice and washed twice using PBS with 1% FCS. To rule out Fc receptor-mediated binding of MG38 to DCs, we verified that the results in the FACS and tissue section studies were not altered by inclusion of 10% human serum in the staining reactions.

Cell Lysates and Metabolic Labeling

Monocytes, T cells, and DCs were harvested, counted, and washed three times with 1 \times PBS, then resuspended in RIPA buffer (150 mM NaCl, 1% NP40, 0.5% DOC, 0.1% SDS, 50 mM Tris-HCl). The following components were added to RIPA buffer before use: 1 mM DTT, 1 mM PMSF and 1 \times proteinase inhibitor cocktail (1 mg/ml leupeptin, 1 mg/ml antipain, 10 mg/ml benzamide hydrochloride, 5 mg/ml aprotinin, 10 mg/ml soy bean trypsin Inhibitor, 1 mg/ml pepstatin). Samples

were rotated at 4°C for 30 min, and nuclei removed by spinning in a microfuge for 10 min. Total protein concentrations of the cell lysate was determined using 1× Bradford Assay buffer reagent (Bio-Rad Laboratories, Hercules, CA, USA). For metabolic labeling, cells were washed once with 1X PBS, then resuspended at 1×10^7 cells per 100 mm plate in 10 ml labeling media (DMEM, without methionine, supplemented with 10% dialyzed fetal bovine serum and antibiotics; Gibco-BRL, Grand Island, NY, USA) and incubated at 37°C for 30 min to allow depletion of the intracellular pools of sulfur-containing amino acids. Cells were then labeled by adding 1 mCi of (35 S) methionine, for 6 h and lysates were prepared as described.

Immunoprecipitation (IP)

Lysates were pre-cleared by incubating with 100 μ l of prewashed 50% protein A Sepharose in the cold for 30–60 min. 0.5–1 μ g of each test antibody was then mixed with 50 μ l of pre-cleared cell lysate and incubated on ice for 1 hr, and 30 μ l of prewashed 50% protein A Sepharose was added for an additional hour. Beads were washed 3 times with cold RIPA buffer and analyzed by SDS-PAGE under reducing conditions.

RESULTS

Isolation of Human DEC-205

We previously reported the isolation of a cDNA clone encoding mouse DEC-205 [1]. By screening both human B cell and thymus libraries with probes derived from the mouse DEC-205 cDNA (see Materials and Methods), we obtained three overlapping cDNAs clones that when combined, encoded the complete human homologue of mouse DEC-205.

The predicted protein encoded by the hDEC-205 cDNAs is a type I transmembrane protein of 1722 amino acids with characteristic features of a group VI C-type lectin. Like mouse DEC-205 the human protein consists of an NH₂-terminal signal peptide followed by a cysteine-rich (CR) domain, a fibronectin type II (FnII) domain, ten carbohydrate recognition-like domains (CRDs), and a transmembrane domain, which is followed by a short cytoplasmic tail. Amino acid sequence alignment between the human and mouse cDNAs [1] reveals 77% identity, suggesting functional conservation. Our huDEC-205 sequence is identical to one independently isolated by Kato *et al.* [8].

Like mouse DEC-205, huDEC-205 is also homologous to other members of the multiple C-type lectin receptors, human macrophage mannose receptor (MMR, 22% identity), a membrane protein implicated in the endocytosis of glycoproteins bearing terminal mannose, fucose, N-acetylglucosamine, or glucose residues [4, 14];

and human phospholipases A₂ (PLA₂) receptor (29% identity), which binds to PLA₂ that specifically catalyzes the hydrolysis of the 2-ester bond of 3-sn-phosphoglycerides [15, 16].

A Monoclonal Antibody Reactive with huDEC-205

Initial attempts to immunize mice with huDEC-205 failed to produce monoclonal antibodies. We reasoned that the failure might be due to the close conservation between human and mouse proteins. To overcome possible cross-tolerance we immunized DEC-knock-out mice. The fusion was initially screened with an ELISA assay against the purified CR-FnII fragment of the immunogen (see Materials and Methods). Reactive monoclonal antibodies were additionally tested by Western blot analysis. Clone MG38 specifically recognized and immunoprecipitated purified CR-FnII but not human Fc (Fig. 2A and B). These results demonstrate that MG38 specifically recognizes an epitope present in the CR-FnII region of human DEC-205. The final step of the screen was to verify MG38 staining of human thymic cortical epithelium, since mouse DEC-205 is abundant on this cell type [5, 6]. MG38 did not react with mouse DCs.

huDEC-205 Is Expressed by LPS Activated Monocyte-derived Dendritic Cells

To determine whether monocyte derived DCs expressed huDEC-205, we performed immunoprecipitation studies. Metabolically labeled monocyte-derived DCs yielded a single band migrating at a molecular weight that was greater than the 205 kDa seen with murine DEC-205 [7] (Fig. 3). A protein with the same molecular weight was also precipitated by rabbit polyclonal antibodies that were raised against mouse DEC-205 (unpublished results and [6, 7]). We presume that the higher apparent molecular weight of human DEC-205 reflects differences due to the primary sequence or posttranslational modifications.

FACS Analysis of DEC-205 Expression

We used FACS analysis to additionally examine expression of huDEC-205 during DC development and maturation (Fig. 4). Immature DCs, mature DCs and macrophages were prepared from monocytes. Surface-bound as well as total DEC-205 was visualized in live and fixed-permeabilized cells, respectively. Other antibodies included a non-reactive IgG2b (termed Y-Ae; isotype-matched to MG38), CD21 (B cell marker), DC-LAMP (DC marker), and CD83 (DC marker). Double labeling was used to identify the DCs as CD14[−] HLA-DR⁺⁺, and CD83⁺.

Only a small fraction of the immature DC preparation expressed high DEC-205 (Fig. 4A; <5% are DEC-205⁺ HLA-DR⁺, as indicated by the arrow). However, the

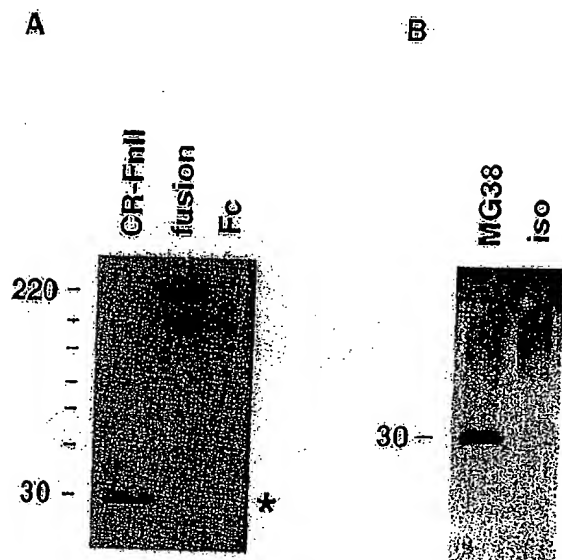


FIGURE 2 MG38 mAb reacts with CR-FnII domains of human DEC-205. (A) 50 ng of the CR-FnII and Fc regions of the fusion protein were separated by SDS-PAGE under non-reducing conditions, transferred to nitrocellulose paper and blotted with MG38. CR-FnII: papain digested and purified CR-FnII region of the fusion protein; fusion: the entire fusion protein; Fc: papain digested and purified Fc region of the fusion protein. MG38 specifically reacted with the CR-FnII protein (*) but not the Fc protein. 2 bands were seen in the fusion protein. The slow-migrating band was the dimer of full-length fusion protein; the fast-migrating band a heterodimer containing one full-length fusion protein and one Fc peptide, which resulted from cleavage of the fusion protein at the junction point during preparation. (B) Immunoprecipitation of CR-FnII by MG38. CR-FnII protein was incubated with MG38 or isotype-matched mAb as a control. The precipitated complexes were separated by SDS-PAGE under reducing conditions and visualized by blotting with rabbit-anti-CR-FnII antiserum. CR-FnII bands are indicated by an asterisk.

number of DEC-205⁺ cells was dramatically increased (>85%) in the mature DC population (Fig. 4A). Staining was increased considerably by permeabilizing the cells with saponin (Fig. 4A). Increased DEC-205 expression was correlated with higher levels of CD83 and DC-LAMP, both antigens being characteristic of mature DCs (Fig. 4B). In contrast, little or no staining was observed in other types of blood leukocytes present in PBMC (not shown). However, EBV-transformed B-LCL did stain with MG38, which is not surprising given the fact that our human DEC-205 cDNA was obtained from a B-LCL library.

Differences in the Expression of MMR and DEC-205 in Sections of Lymphoid Tissue

Because monoclonals to human MMR had been prepared previously [3], we were in a position to use monoclonals

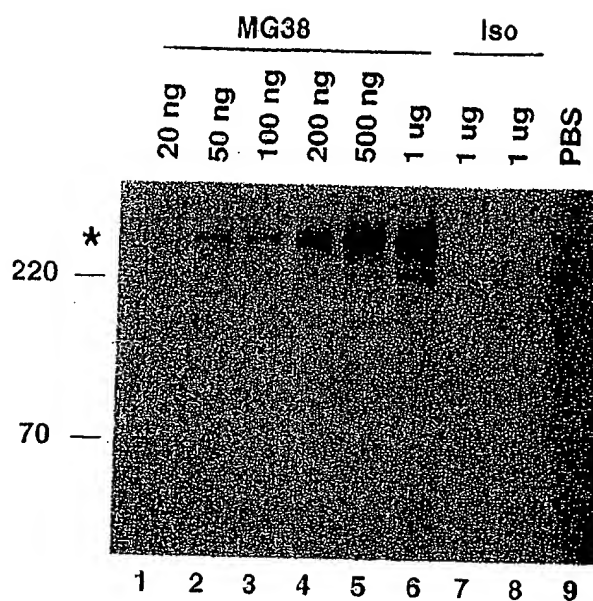


FIGURE 3 Metabolic labeling of DEC-205. Lysates from metabolically-labeled mature DCs were incubated with MG38 in amounts ranging from 20 ng to 1 μ g (lane 1 to lane 6). Increasing amounts of DEC-205 (*) were immunoprecipitated with increasing amounts of MG38, saturating at a level of 500 ng of MG38. The DEC-205 band was not obtained when using a control isotype-matched antibody (lane 7 and 8) or PBS (without Ab, lane 9).

to compare the *in vivo* expression of MMR with DEC-205. We studied sections of human tonsil and thymus. In tonsil, presumptive DCs in the T cell areas stained for DEC-205 (Fig. 5A, 5B). In contrast, the MMR was found on scattered presumptive macrophages in the septae; when found near the T cell area, the MMR was clearly present on vascular profiles, presumably efferent lymphatics (arrow, Fig. 5D). The latter originate in the T cell regions to carry tonsillar lymphocytes to the cervical and other lymph nodes in the neck (Fig. 5C, 5D). Neither DEC-205 nor MMR were found on blood vessels.

In thymus, DEC-205 stained the cortical epithelium (red stain in Fig. 6 left) and large dendritic profiles in the thymic medulla (red stain in Fig. 6 right). The latter medullary profiles double labeled for the DC-restricted marker DC-LAMP (not shown). In contrast, the MMR was found on scattered cells, presumably macrophages, in the thymic cortex and septae (green stain in Fig. 6 left) and on infrequent profiles in the medulla (green stain in Fig. 6 right). Two color immunofluorescence of thin 1 micron optical sections, examined by deconvolution microscopy, clearly showed that DEC-205 and MMR were expressed at different sites in the thymus. Therefore, these two homologous multilectins are expressed in dif-

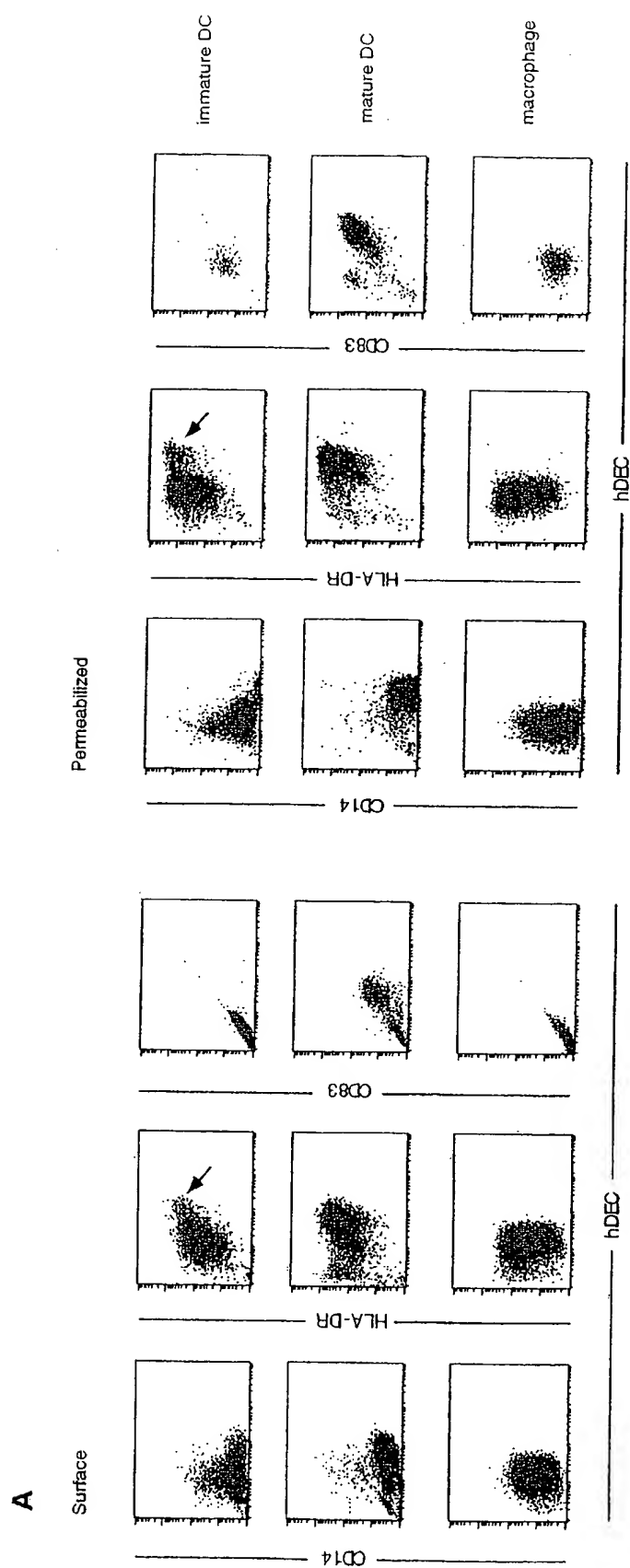
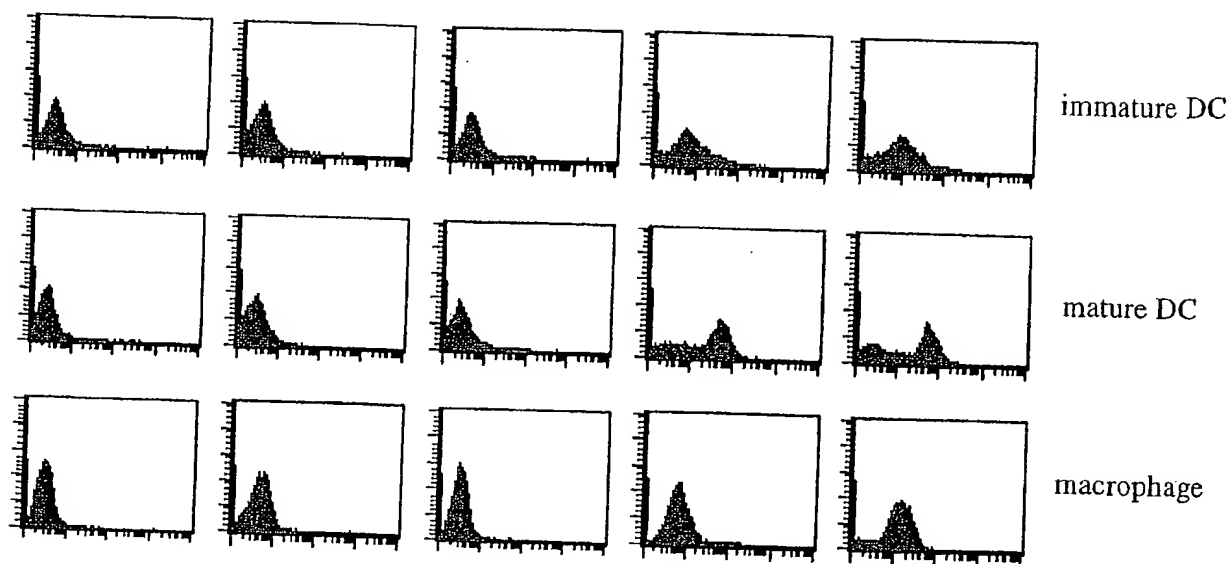


FIGURE 4 (A) DEC-205 is induced in mature DCs. Flow cytometry on live (surface) or saponin-permeabilized monocyte-derived macrophages, immature DCs, and mature DCs. Double staining of cells with MG38, detected by FITC-goat-anti-mouse Ig, and PE-antibodies: PE-CD14, PE-HLA-DR, and PE-CD83. A small subset of mature DEC-205⁺ HLA-DR⁺ cells in the immature DC preparation is arrowed.

B

Surface



Permeabilized

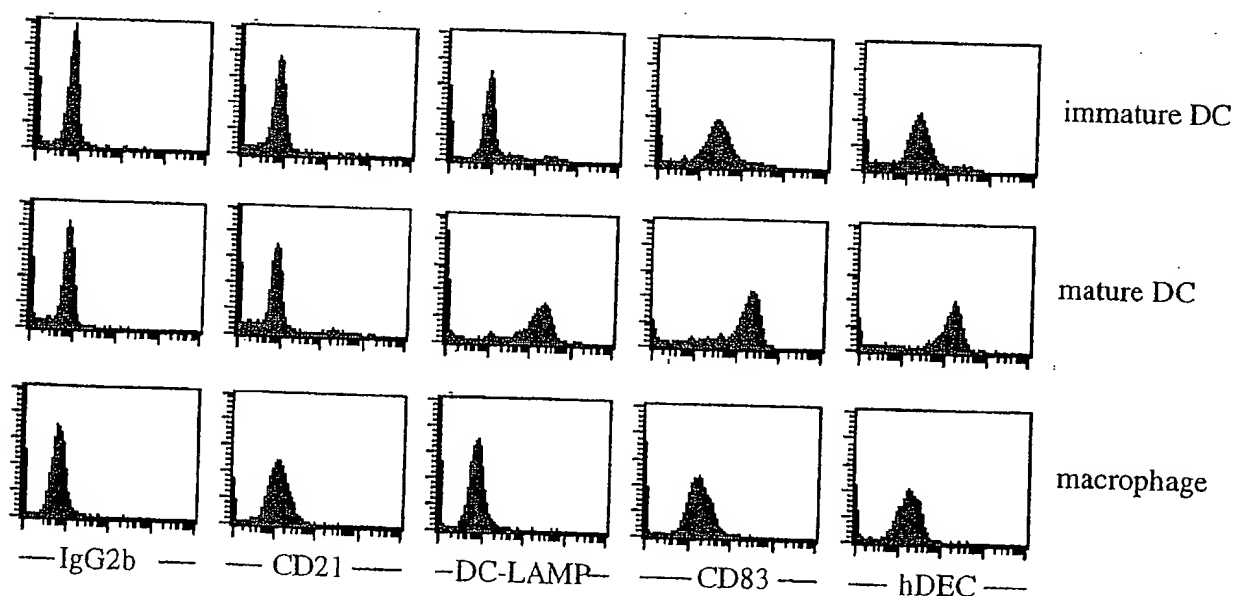


FIGURE 4 (B) DEC-205 is induced in mature DCs. Flow cytometry on live (surface) or saponin-permeabilized monocyte-derived macrophages, immature DCs, and mature DCs. Histograms of DEC-205, CD83, DC-LAMP, and CD21 expression. Mature DCs, but not immature DCs or macrophages, expressed high levels of DEC-205, CD83 and DC-LAMP.

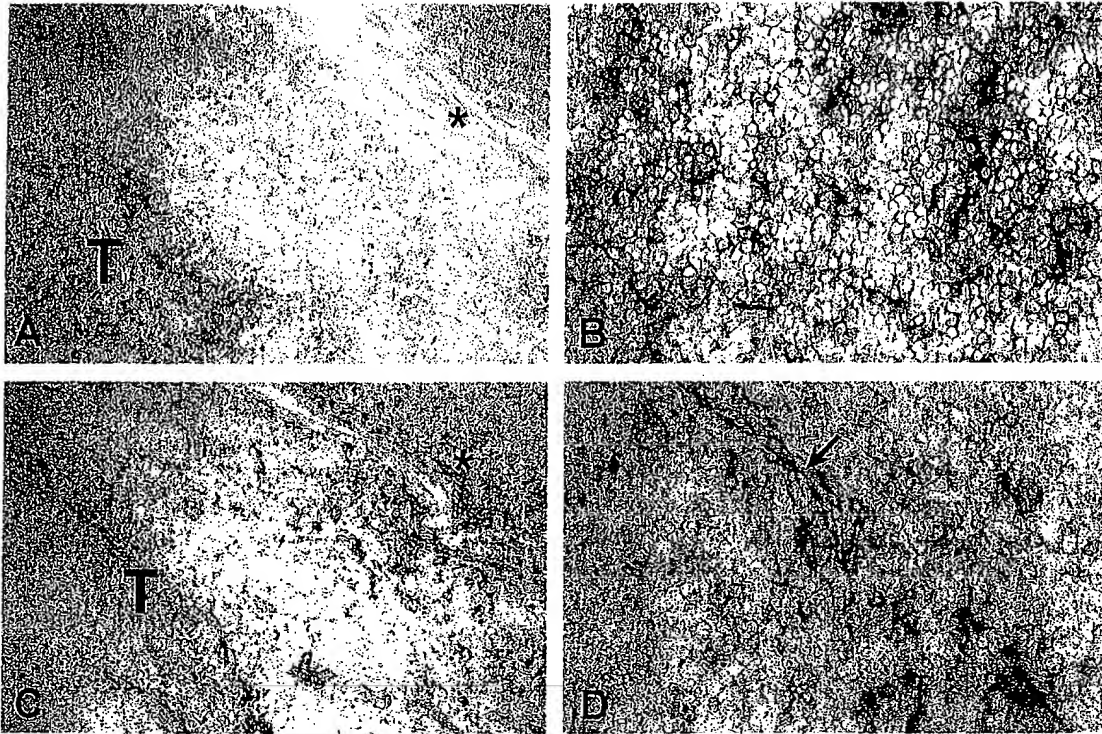


FIGURE 5 Expression of human DEC-205 and MMR in select regions of human tonsil. At low power (A,C), a T cell area (left) is next to a vessel rich (*) nonparenchymal region (right); at high power, a T cell area is shown (B,D). The sections were labeled in brown for CD8 to identify T cells and in blue for DEC-205 (MG 38; panels A,B) or MMR (mAb 3.29; panels C,D). The MMR, when found in the T cell area, is on lymphatic vessels (D, arrow), while in nonparenchymal regions, MMR is found on scattered cells, probably macrophages (C). DEC-205 is found on DCs in the T cell area (B).

ferent sites in lymphoid tissues, and only DEC-205 is abundant on DCs.

DISCUSSION

The availability of monoclonal antibodies to human MMR and DEC-205 now makes it possible to look more closely at the tissue distribution of these homologous, multilectin receptors for adsorptive endocytosis. A single monoclonal has been available to mouse DEC-205 but only polyclonals and antisense RNA probes for the mouse MMR. For human, several monoclonals to the MMR have been produced but none to human DEC-205. We have been able to secure the MG38 monoclonal to human DEC-205 by immunizing DEC-205 knockout mice with the NH₂-terminal region of the recently cloned human DEC-205. The MG38 antibody reacts with the immunogen produced from DEC-205 cDNA, it

precipitates a 205 kD polypeptide from metabolically labeled mature DCs, and it stains the thymic cortical epithelium much like the rat anti-mouse DEC-205 antibody. On isolated human blood cells, the expression of DEC-205 is similar to what is known for mouse DEC-205 [5, 7, 17]. The extent of analysis in the human system is still limited, but DEC-205 is expressed on DCs derived from blood monocytes, while PBMCs stain weakly or not at all with MG38. The presence of weak FACS signals in other leukocytes is in keeping with the fact that our cDNA was derived from a human lymphocyte library.

In tissue sections of tonsil and thymus, DEC-205 and MMR are expressed on different cells. As in the mouse, MG38 stains large dendritic profiles in the T cell areas of tonsil and the medulla of the thymus, as well as the thymic epithelium. In contrast, the MMR is found on scattered macrophages in the trabeculae or septae of both organs, and on efferent lymphatic endothelium in the tonsil. Recently [18], it has been reported that the mouse MMR is often expressed on lymphatic vessels in mouse lymphoid tissues, but not in the T cell areas, a known site for the localization of DCs. Two other studies have shown that a polyclonal antibody to the mouse MMR also stains sinusoidal lining cells in lymph node and liver, but does not stain DCs in the T cell areas [18, 19]. In sum, even though MMR and DEC-205 are homologous in structure and expressed on monocyte-derived

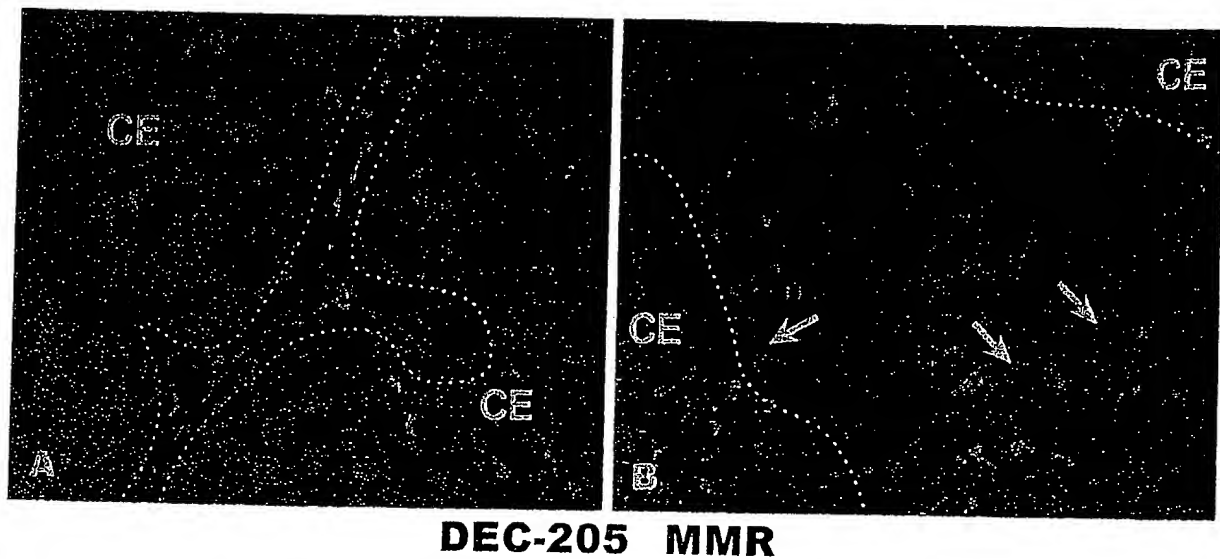


FIGURE 6 Expression of DEC-205 (red) and MMR (green) in the thymic cortex (A) and medulla (B) using two color indirect immunofluorescence and deconvolution of 0.5 μ sections (see Materials and Methods). A septum in the thymic cortex (A) and the thymic medulla (B), are outlined with white dots. The two receptors are expressed in different cells: scattered, green macrophages for the MMR, and the cortical epithelium and medullary DCs (arrows) for DEC-205.

DCs, the tissue distribution of these two endocytosis receptors is quite different.

Because both the MMR and DEC-205 are found on DCs produced *in vitro* from human monocytes with GM-CSF and IL-4, it is possible that both are involved in the presentation of foreign antigens. Recently, it has been shown that blood derived mouse monocytes can capture particles in a peripheral extravascular site and then enter the lymph and T cell areas of the draining lymph node to become DCs [20]. Monoclonals are not yet available to verify the expression of mouse MMR and DEC-205 on monocyte-derived DCs *in vivo*. However, in the steady state, the MMR and DEC-205 are expressed in different sites in lymphoid tissues and may have distinct functions. We speculate that a biological role of the MMR in sinusoidal endothelium is to clear self proteins that have been altered to expose mannosyl residues. In contrast, DEC-205 on thymic epithelium and DCs in the thymic medulla and T cell areas may function to capture self proteins for thymic and peripheral selection events.

ACKNOWLEDGMENTS

The authors thank Dr. Angela Granelli-Piperno for providing leukocytes used for the FACS studies; Dr. William Muller for securing specimens of human thymus and tonsil that would

otherwise have been discarded; and Drs. Antonio Lanzavecchia, Sem Saeland and Serge Lebecque for generously providing monoclonals.

REFERENCES

1. Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM, Nussenzweig MC: The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 375:151, 1995.
2. Maurer D, Fiebiger E, Reininger B, Ebner C, Petzelbauer P, Shi G-P, Chapman HA, Stingl G: Fc ϵ receptor I on dendritic cells delivers IgE-bound multivalent antigens into a cathepsin S-dependent pathway of MHC class II presentation. *J Immunol* 161:2731, 1998.
3. Sallusto F, Cella M, Danieli C, Lanzavecchia A: Dendritic cells use macropinocytosis and the mannose receptor to concentrate antigen in the major histocompatibility class II compartment. Downregulation by cytokines and bacterial products. *J Exp Med* 182:389, 1995.
4. Engering AJ, Cella M, Fluitsma D, Brockhaus M, Hoefsmit EC, Lanzavecchia A, Pieters J: The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur J Immunol* 27: 2417, 1997.
5. Kraal G, Breel M, Janse M, Bruin G: Langerhans' cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. *J Exp Med* 163:981, 1986.
6. Witmer-Pack MD, Swiggard WJ, Mirza A, Inaba K, Steinman RM: Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. II. Expression in situ in lymphoid and nonlymphoid tissues. *Cell Immunol* 163:157, 1995.
7. Inaba K, Swiggard WJ, Inaba M, Meltzer J, Mirza A, Sasagawa T, Nussenzweig MC, Steinman RM: Tissue

- distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse leukocytes. *Cell Immunol* 163:148, 1995.
8. Kato M, Neil TK, Clark GJ, Morris CM, Sorg RV, Hart DNJ: cDNA cloning of human DEC-205, a putative antigen-uptake receptor on dendritic cells. *Immunogenetics* 47:442, 1998.
 9. Mizushima S, Nagata S: PEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Res* 18:5322, 1990.
 10. Harlow E, Lane D: *Antibodies: A Laboratory Manual*. New York, Cold Spring Harbor Laboratory, 1988.
 11. Gong S, Nussenzweig MC: Regulation of an early developmental checkpoint in the B cell pathway by IgE. *Science* 272:411, 1995.
 12. Granelli-Piperno A, Delgado E, Finkel V, Paxton W, Steinman RM: Immature dendritic cells selectively replicate M-tropic HIV-1, while mature cells efficiently transmit both M- and T-tropic virus to T cells. *J Virol* 72:2733, 1998.
 13. de Saint-Vis B, Vincent J, Vandenabeele S, Vanbervliet B, Pin J-J, Ait-Yahia S, Patel S, Mattei M-G, Banchereau J, Zurawski S, Davoust J, Caux C, Lebecque S: A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment. *Immunology* 9:325, 1998.
 14. Harris N, Super M, Rits M, Chang G, Ezekowitz RA: Characterization of the murine macrophage mannose receptor: demonstration that the downregulation of receptor expression mediated by interferon-gamma occurs at the level of transcription. *Blood* 80:2363, 1992.
 15. Ishizaki J, Hanasaki K, Higashino K, Kishino J, Kikuchi N, Ohara O, Arita H: Pancreatic-type phospholipase A2 induces group II phospholipase A2 expression and prostaglandin biosynthesis in rat mesangial cells. *J Biol Chem* 269:5897, 1994.
 16. Higashino K, Ishizaki J, Kishino J, Ohara O, Arita H: Structural comparison of phospholipase-A2-binding regions in phospholipase-A2 receptor from various mammals. *Eur J Biochem* 225:375, 1994.
 17. Swiggard WJ, Mirza A, Nussenzweig MC, Steinman RM: DEC-205, a 205 kDa protein abundant on mouse dendritic cells and thymic epithelium that is detected by the monoclonal antibody NLDC-145: Purification, characterization and N-terminal amino acid sequence. *Cell Immunol* 165:302, 1995.
 18. Linehan SA, Martinez-Pomares L, Stahl PD, Gordon S: Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: In situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. *J Exp Med* 189:1961, 1999.
 19. Takahashi K, Donovan MJ, Rogers RA, Ezekowitz RA: Distribution of murine mannose receptor expression from early embryogenesis through to adulthood. *Cell Tissue Res* 292:311, 1998.
 20. Randolph GJ, Inaba K, Robbani DF, Steinman RM, Muller WA: Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunology* 11:753, 1999.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.